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Redescription, DNA barcoding, and new distributional records of *Mordellistena microgemellata* Ermisch, 1965 (Coleoptera, Mordellidae)

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Abstract

Mordellistena microgemellata Ermisch, 1965, recorded from Bulgaria, Cyprus, Greece, and Hungary, is here reported for the first time from Tunisia. A detailed redescription of the species, supplemented with photographs and illustrations of key diagnostic characters, is provided based on the examination of the holotype and 25 additional specimens. Furthermore, six sequences of the DNA barcoding fragment of the cytochrome c oxidase I (COI) gene generated in this study represent the first available DNA data for the species.

Key Words

Cytochrome c oxidase, holotype, phylogeny, tumbling flower beetles, Tunisia

Introduction

The fauna of Tunisia is currently known to comprise 17 species of Mordellidae (Horák 2020). In this study, we add *Mordellistena microgemellata* Ermisch, 1965 to this list. The species was originally described based on two specimens collected in Greece, without further locality details (Ermisch 1965). Since its description, *M. microgemellata* has been mentioned only four times in the literature – in the identification keys to Central European *Mordellistena* by Ermisch (1977) and Kaszab (1979), and in the distributional catalogues of Horák (2008, 2020), where it was reported for the first time from Bulgaria, Cyprus, and Hungary. The original description was brief and limited to male characters, which has prevented reliable separation of *M. microgemellata* from the morphologically similar *M. psammophila* Peyerimhoff, 1943, described from Algeria and known only from two female syntypes. Moreover, no DNA sequences of *M. microgemellata* have been available to date.

During a collecting trip to Tunisia in 2025, the first author sampled 25 specimens identified as *M. microgemellata* through direct comparison with the male holotype. This material allowed us to provide a detailed redescription of the species, including female characters that enable reliable separation from *M. psammophila*. The newly collected material also yields the first DNA barcodes for *M. microgemellata*, which are now publicly available in the Barcode of Life Data System (BOLD) and GenBank.

Material and methods

We examined the male holotype of *M. microgemellata* and 25 additional specimens collected in Tunisia in 2025. For comparative purposes, we also studied the primary types of the following species: *M. algeriensis* Ermisch, 1966 (SNSD); *M. aureotomentosa* Ermisch, 1977 (SNSD); *M. elbrusicola* Ermisch, 1969 (SNSD); *M. gemellata* Schilsky, 1899 (MNB); *M. maroccana* Ermisch, 1966 (SNSD); *M. microgemellata* Ermisch, 1965 (SNSD); *M. peloponnesensis* Batten, 1980 (NBCL); *M. psammophila* Peyerimhoff, 1943 (MNHN); *M. pyrenaea* Ermisch, 1966 (SNSD); *M. rhenana* Ermisch, 1956 (SNSD); *M. wankai* Ermisch,

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1966 (SNSD); and *M. zoltani* Ermisch, 1977 (HNHM). The examined material is deposited in the following private collections and institutions: Dávid Selnekovič private collection, Bratislava, Slovakia (DSBS); Hungarian Natural History Museum, Budapest, Hungary (HNHM); Naturalis Biodiversity Centre, Leiden, The Netherlands (NBCL); Museum für Naturkunde der Humboldt-Universität, Berlin, Germany (NMB); Muséum national d'Histoire naturelle, Paris, France (MNHN); Senckenberg Naturhistorische Sammlungen, Dresden, Germany (SNSD).

All examined specimens, including types and DNA-voucher specimens, were soaked for several hours in 5% acetic acid at room temperature, dissected, and mounted on cards. Dissected body parts used for drawings and photographs were cleared in 5% KOH at room temperature for several days and then mounted on temporary slides in glycerol. After examination, these body parts were mounted on the card together with the respective specimen using dimethyl hydantoin formaldehyde (Entomopraxis, Barcelona, Spain). Specimens were studied under a Leica M205 C stereomicroscope (Leica, Wetzlar, Germany) with magnification up to 160× and diffused LED light (6400 K). Habitus photographs were taken with a Canon EOS 5D Mark II camera (Canon, Tokyo, Japan) attached to a Zeiss Axio Zoom.V16 stereoscope (Zeiss, Oberkochen, Germany), and photographs of dissected body parts were taken with a Zeiss Axio Imager.M2 microscope. Images were stacked using Zerene Stacker v.1.4 (https://zerenesystems.com/cms/stacker) and edited with Adobe Photoshop CC (https://www.adobe.com/products/photoshop.html). Drawings of genitalia were prepared using a drawing tube attached to a Leica DM1000 microscope. Measurements were taken with an ocular micrometer in the Leica M205 C stereomicroscope. Values are given as ranges, followed by the arithmetic mean and standard deviation in parentheses. The measured characters are abbreviated as follows: EL – elytral length from scutellar apex to elytral apices along suture; EW – maximum elytral width; HL – head length from anterior clypeal margin to occipital carina along midline; HW – maximum head width; LPrL – maximum left paramere length; PL – pronotal length along midline; PW – maximum pronotal width; PygL – maximum pygidial length; RPrL – maximum right paramere length; TL – combination of head, pronotal and elytral lengths. Ocular index refer to a ratio of head width and minimum interocular distance.

DNA isolation and amplification procedures followed Selnekovič et al. (2023). Barcoding fragments of the COI gene were amplified by PCR using the standard primer pair LEP-F1 and LEP-R1 (Hebert et al. 2003). Nucleotide sequences were trimmed and assembled into contigs with ChromasPro v.2.1.10 (Technelysium Pty Ltd, South Brisbane, Queensland, Australia). The final alignment was performed manually in Mesquite v.3.81 (Maddison and Maddison 2023). The initial dataset consisted of newly generated sequences of *M. microgemellata* together with all sequences of *Mordellistena* from Europe and North Africa available on BOLD. Unique haplotypes were filtered using the FaBox DNA to haplotype collapser tool (Villesen 2007), and the collapsed dataset was used for subsequent analyses. BOLD Process IDs and GenBank accession numbers of all sequences used in this study are provided in the Suppl. material 1.

Uncorrected pairwise distances (p-distances) were calculated in MEGA v.11 (Tamura et al. 2021). All ambiguous sites were coded as missing prior to the computation. Maximum likelihood (ML) analysis was performed in IQ-TREE (Nguyen et al. 2015). The best-fit substitution model (GTR+F+R4) was selected by the built-in ModelFinder using the Bayesian Information Criterion. Node support was assessed with 1000 ultrafast bootstrap replicates and the SH-like aLRT test (Guindon et al. 2010), also with 1000 replicates. The resulting tree was rooted and edited in Interactive Tree of Life (iTOL) v.6 (Letunic and Bork 2024). *Tomoxia bucephala* Costa, 1854 and *Mordella aculeata* Linnaeus, 1758 (Mordellidae, Mordellini) were used as outgroups.

Results

Species redescription

Mordellistena microgemellata Ermisch, 1965

Figs 1–3

Mordellistena microgemellata Ermisch, 1965: 265–267; Ermisch 1977: 163; Kaszab 1979: 40–41; Horák 2008: 99; Horák 2020: 96.

Material examined. ***Holotype*** (designated in original description) Greece • male; "Graecia"; SNSD [genitalia dissected]. Photograph of the holotype available at https://doi.org/10.5281/zenodo.17435622.

Other material. Tunisia • 19 males, 6 females; Bir Al Huffay; 34.908249°N, 9.179063°E; alt. 435 m; 11 May 2025; D. Selnekovič leg.; ruderal vegetation, on *Anethum*; DSBS, DSBS1079 to DSBS1083, DSBS1088.

Differential diagnosis. *Mordellistena microgemellata* was included in the *M. gemellata* morphological species group (Ermisch 1965) based on the following features: antennomeres 5–10 distinctly longer and broader than the preceding two segments, and a metatibia bearing, in addition to the apical ctenidium, two short lateral ctenidia oriented approximately perpendicular to the dorsal tibial margin.

Within the *M. gemellata* group, *M. microgemellata* is further characterised by its entirely black integument, including the metatibial spurs; pale vestiture (yellowish to brownish); uniformly coloured elytral vestiture, with only a narrow, darkened band along the suture; and short antennomeres 5–10, each up to 1.25× longer than wide (Fig. 2A). The relative length of antennomeres separates the species from *M. gemellata* (Portugal, Spain), in which antennomeres 5–10 are about 2.0× longer than wide.

Mordellistena microgemellata differs from *M. algeriensis* (Algeria, Italy, Tunisia), *M. aureotomentosa* (Morocco), *M. peloponnesensis* (Cyprus, Greece, Italy, Turkey), and *M. pyrenaea* (Spain) by the penultimate abdominal tergite

being concealed by the elytra (Fig. 1) (in the former species it is usually exposed). In addition, the male sternite VIII of *M. microgemellata* has broadly rounded and indistinct postero-lateral angles (Fig. 2H), whereas in the former species these angles are prominent (Selnekovič and Kodada 2022; Selnekovič et al. 2024). Each of these species also differs from *M. microgemellata* in the shape of the parameres [see Selnekovič and Kodada (2022) for *M. algeriensis*; Selnekovič et al. (2024) for *M. peloponnesensis*; Ermisch (1966a) for *M. pyrenaea*; Ermisch (1966b) for *M. aureotomentosa*]. *Mordellistena maroccana* (Morocco, Tunisia) differs from *M. microgemellata* in having a wider body, with shorter and wider elytra.

The most similar species is *M. psammophila*, known only from two female syntypes from Algeria (Peyerimhoff 1943). Females of *M. microgemellata* can be reliably separated by the structure of sternite VIII: in *M. microgemellata* the posterior margin is produced and bears long trichoid sensilla, which are nearly as long as the sternite width (Fig. 2I), while in *M. psammophila* the homologous sensilla are much shorter (Fig. 2J). Furthermore, the spiculum ventrale is longer and clavate basally in *M. microgemellata* (ca. 0.7× as long as sternite along midline), but shorter and cylindrical in *M. psammophila* (ca. 0.4×).

The sympatric *M. pseudohirtipes* Ermisch, 1965 may also resemble *M. microgemellata*, but it can be distinguished by having at least three well-developed lateral metatibial ctenidia and differently shaped parameres (Selnekovič and Kodada 2019).

Description. Dimensions (mm; males n = 10, females n = 4): TL: ♂♂ 3.14–3.62 (3.40 ± 0.16), ♀♀ 3.14–3.41 (3.32 ± 0.12); HL: ♂♂ 0.61–0.69 (0.65 ± 0.02), ♀♀ 0.60–0.66 (0.63 ± 0.03); HW: ♂♂ 0.65–0.76 (0.70 ± 0.03), ♀♀ 0.61–0.67 (0.65 ± 0.03); PL: ♂♂ 0.78–0.91 (0.85 ± 0.04), ♀♀ 0.76–0.83 (0.81 ± 0.03); PW: ♂♂ 0.77–0.92 (0.86 ± 0.05); ♀♀ 0.78–0.86 (0.84 ± 0.04); EL: ♂♂ 1.76–2.05 (1.90 ± 0.11), ♀♀ 1.79–1.94 (1.89 ± 0.07); EW ♂♂ 0.85–0.95 (0.91 ± 0.03), 0.82–0.95 (0.91 ± 0.06); PygL: ♂♂ 1.01–1.14 (1.09 ± 0.04), ♀♀ 0.88–0.97 (0.94 ± 0.04); RPrL: 0.25–0.30 (0.27 ± 0.01); LPrL: 0.31–0.36 (0.34 ± 0.02).

Body elongate, wedge-shaped (Fig. 1); widest at middle of pronotum in males and before midlength of elytra in females. Integument entirely black. Vestiture yellowish with a weak reddish to purplish lustre, slightly darkened in a narrow band along the elytral suture, and dark brownish to black on the posterior portions of ventrites 3 and 4, the whole of ventrite 5, and the pygidium.

Head moderately convex, highest point situated behind the middle in lateral view; HW/HL ratio: ♂♂ 1.03–1.14 (1.07 ± 0.03), ♀♀ 1.02–1.06 (1.04 ± 0.02). Anterior clypeal margin straight. Occipital carina evenly convex. Surface finely microreticulate, with round setiferous punctures. Labrum transverse, setose, shallowly emarginate at anterior margin. Eyes oval, about 1.6× longer than wide, finely faceted, setose; interfacetal setae longer than facet diameter; ocular index: ♂♂ 1.44–1.60 (1.52 ± 0.05), ♀♀ 1.46–1.55 (1.49 ± 0.04). Postocular gena explanate, slightly narrower than facet diameter, not angulate. Antenna weakly serrate (Fig. 2A); scapus and pedicel cylindrical, subequal in width, pedicel slightly longer; antennomere 3 smallest, conical, slightly shorter and narrower than following; antennomere 4 conical, about 0.7× as long as following and slightly narrower; antennomeres 5–10 subtrapezoidal, 1.20–1.25× longer than wide in both sexes; antennomere 11 oval, about 2.0× longer than wide. Galea moderately long, not produced (Fig. 2B, C). Maxillary palpomere 2 strongly expanded with long trichoid sensilla on ventral surface in males (Fig. 2B), not expanded and without markedly long setae in females (Fig. 2C); terminal palpomere triangular, about 2.2× longer than wide, with inner angle at midlength in males, slenderer and with inner angle behind midlength in females. Labial terminal palpomere cylindrical. (Fig. 2D)

Pronotum moderately convex, widest at middle; PW/PL ratio: ♂♂ 0.99–1.07 (1.02 ± 0.03), ♀♀ 1.01–1.06 (1.04 ± 0.02); anterior margin weakly produced at middle; lateral carina concave in lateral view; postero-lateral angles rectangular, narrowly rounded in lateral view. Surface microreticulate, with rasp-like setiferous punctures. Scutellum triangular. Metanepisternum subtrapezoidal, dorsal margin concave, ventral margin straight, posteriorly about as wide as mesotibia.

Elytra moderately convex; EL/EW ratio: ♂♂ 1.97–2.22 (2.10 ± 0.08), ♀♀ 2.03–2.18 (2.07 ± 0.07); lateral margins rounded, evenly converging (Fig. 1); surface strongly microreticulate, with very dense rasp-like setiferous punctures.

Protrochantin with extended sensillum only slightly longer and thicker than surrounding setae, which are distinctly longer in males than females (Fig. 2E, F). Profemur uniformly setose, without conspicuous extended sensilla; setae distinctly longer in males than in females (Fig. 2E, F). Protibia about as long as protarsus; in males weakly expanded behind base and bearing a distinct fringe of extended setae (Fig. 2E), in females straight, without extended setae (Fig. 2F). Protarsomeres cylindrical; in males with several thick extended setae ventro-laterally. Protarsomere 1 slightly longer than next two combined; protarsomere 3 not expanded, apical margin weakly concave. Mesotrochantin with distinct extended sensillum. Mesotibia about 0.8× as long as mesotarsus. Metatibia with two short lateral ctenidia reaching to midwidth of tibia, sometimes with trace of a third ctenidium (Fig. 2G); apical spurs dark brown to black, inner spur about 1.6× as long as outer one. Metatarsomere 1 with three ctenidia; metatarsomere 2 with two (Fig. 2G).

Pygidium acuminate, not constricted (Fig. 1); EL/PygL ratio: ♂♂ 1.65–1.87 (1.75 ± 0.07), ♀♀ 1.96–2.07 (2.02 ± 0.05). Ventrite 5 with apical margin convex. Sternite VIII in males about 1.5× longer than wide, with posterior margin produced and bearing long trichoid sensilla (Fig. 2H); in females posterior margin produced, with markedly long trichoid sensilla along lateral and posterior margins, being nearly as long as sternite width (Fig. 2I); spiculum ventrale clavate, about 0.7× as long as sternite midline.

Phallobase long (Fig. 2K), 0.5× as long as EL; epimere sclerotised, lateral margins weakly concave. Left paramere long (Fig. 3); dorsal process strongly expanded, obliquely truncate apically; median process short and rounded; ven-

Figure 1. *Mordellistena microgemellata* Ermisch, 1965 habitus. **A.** Male, DSBS1080; **B.** Female.

tral process short and weakly curved dorsad; five to seven campaniform sensilla in longitudinal row situated near base of dorsal process. Right paramere long (Fig. 3); dorsal process moderately expanded, rounded apically; ventral process slightly shorter than dorsal one, shallowly bifurcate apically. Penis long, weakly expanded before apex.

Ovipositor with paraprocts short (Fig. 2L); coxite about 1.3× as long as paraproct, with three strongly extended lateral sensilla; stylus with two apical trichoid sensilla; proctiger wide, apex truncate.

Secondary sexual dimorphism. Males are slightly more slender than females (Fig. 1). The second maxillary palpomere is expanded and bears strongly extended setae in males (Fig. 2B) but is unexpanded and lacks extended setae in females (Fig. 2C); in addition, the male fourth (apical) maxillary palpomere is substantially wider than that of the female. The protrochantin and profemur carry distinctly longer setae in males (Fig. 2E) than in females (Fig. 2F). The protibia is expanded behind the base and bears a distinct fringe of extended setae in males (Fig. 2E), while it is straight and without extended setae in females (Fig. 2F). The pygidium is shorter in females.

DNA sequences. Six partial sequences of the COI gene are available on BOLD and GenBank. For accession numbers refer to Suppl. material 1.

Distribution. Bulgaria, Cyprus, Greece, Hungary, Tunisia (new record).

Results of DNA analyses

Six COI barcoding sequences of *M. microgemellata* were generated in this study. All specimens originated from a single locality in Bir Al Huffay, Tunisia. The sequences represented two haplotypes separated by a single substitution (position 340: C/T), corresponding to a 0.15% p-distance.

Figure 2. *Mordellistena microgemellata* Ermisch, 1965. **A.** Antenna, male DSBS1079 (left), female (right); **B.** Maxilla, male DSBS1079; **C.** Maxilla, female; **D.** Labium, male DSBS1079; **E.** Foreleg, male DSBS1079; **F.** Foreleg, female; **G.** Hindleg, male DSBS1079; **H.** Sternite VIII, male DSBS1079; **I.** Sternite VIII, female; **K.** Phallobase, DSBS1079; **L.** Ovipositor. *Mordellistena psammophila* Peyerimhoff, 1943; **J.** Sternite VIII, female, syntype EC33082.

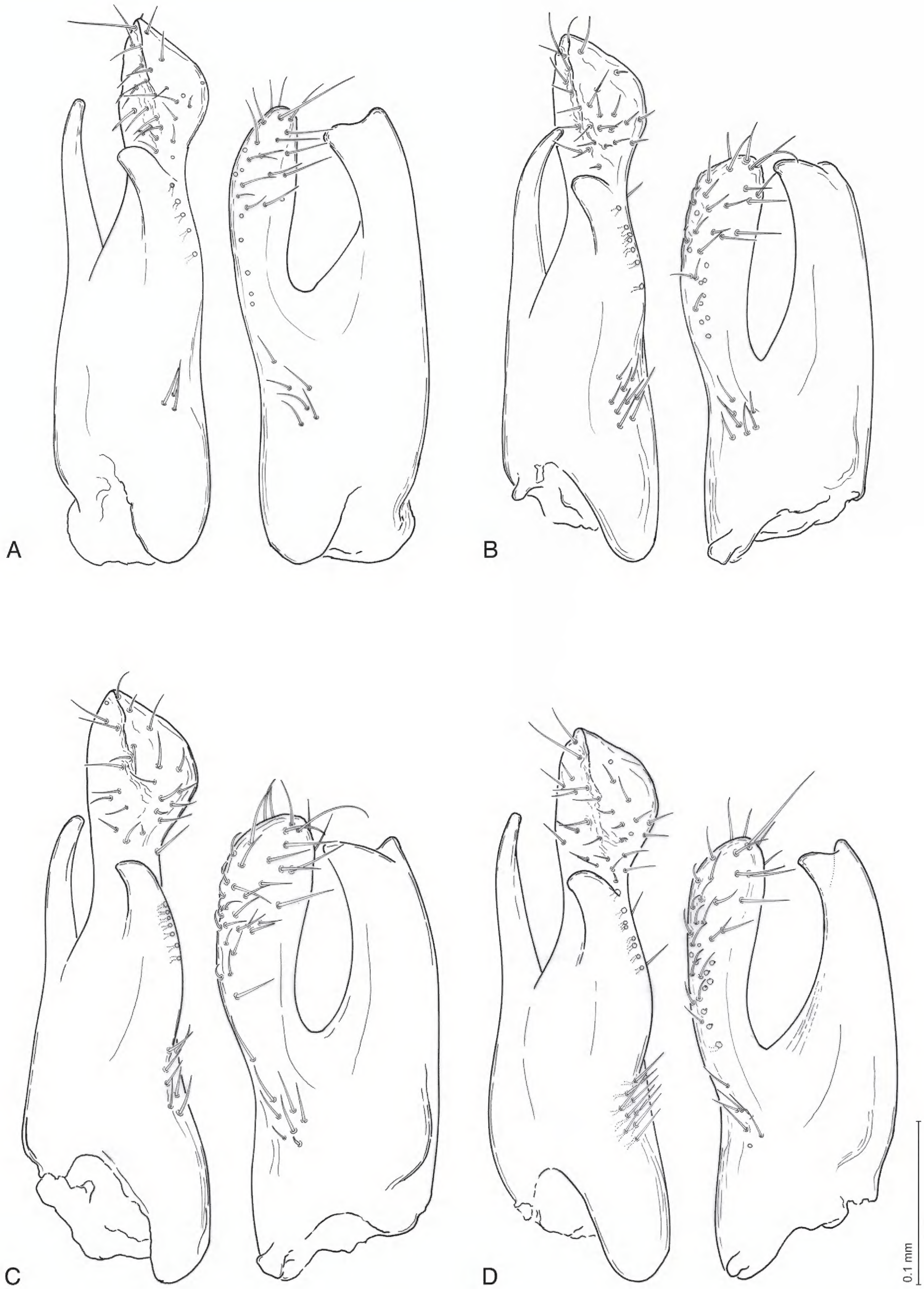
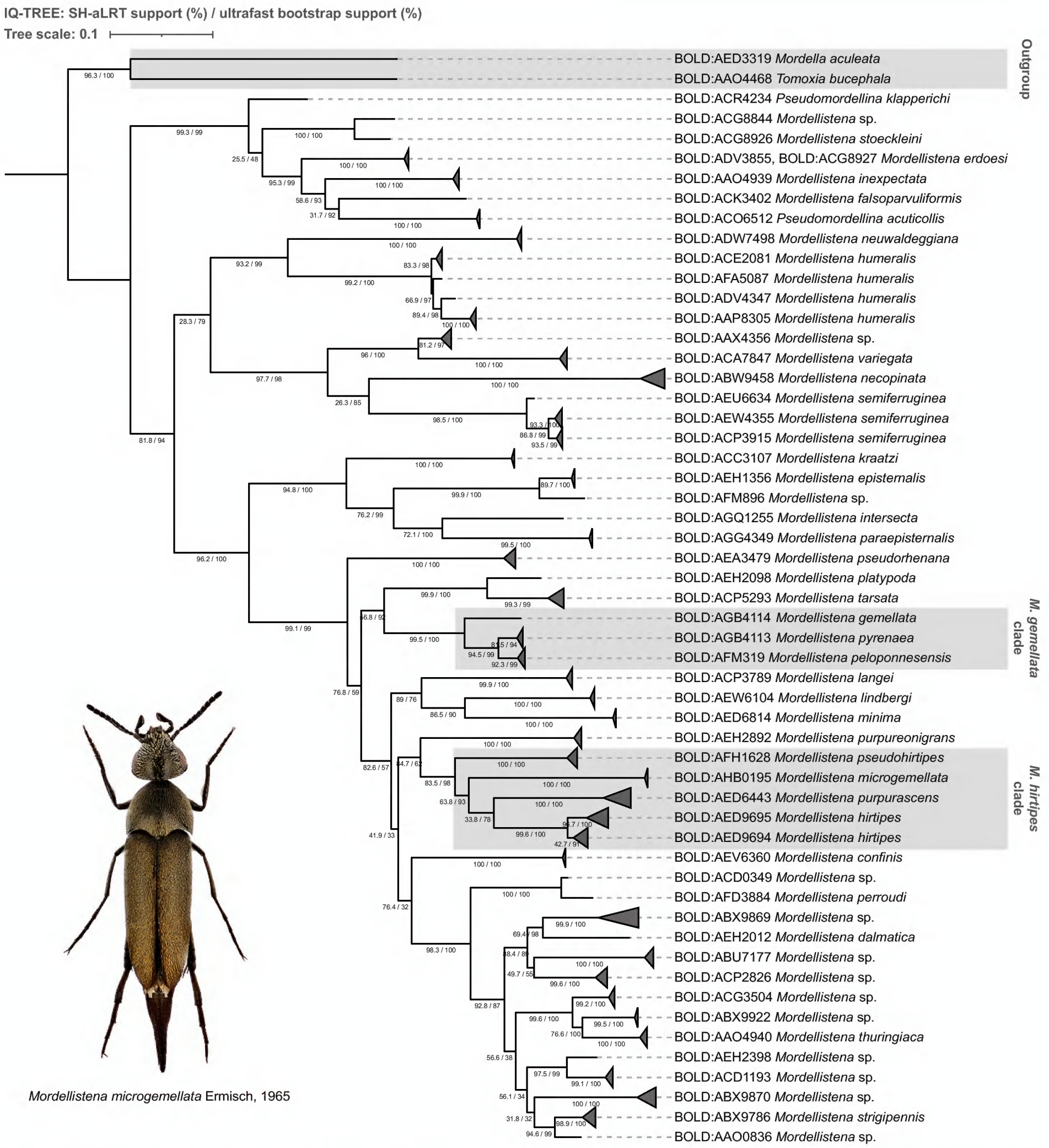


Figure 3. *Mordellistena microgemellata* Ermisch, 1965 parameres. **A.** Holotype; **B.** DSBS1081; **C.** DSBS1080; **D.** DSBS1079. Scale bar: 0.1 mm.



Mordellistena microgemellata Ermisch, 1965

Figure 4. Phylogenetic tree based on publicly available sequences of *Mordellistena* from Europe and North Africa. The tree was inferred from 259 unique haplotypes of a 658 bp COI fragment using the maximum likelihood method in IQ-TREE. Node support is indicated by ultrafast bootstrap and SH-aLRT values. Each terminal clade corresponds to a unique Barcode Index Number (BIN) assigned in the Barcode of Life Database (BOLD).

The nearest neighbour within the dataset was *M. hirtipes* Schilsky, 1895, with interspecific p-distances ranging from 14% to 15.22%. Maximum likelihood analysis revealed that *M. microgemellata* is not closely related to members of the *M. gemellata* species group (*M. gemellata*, *M. peloponnesensis*, *M. pyrenaea*), but instead clusters within the *M. hirtipes* species group (*M. hirtipes*, *M. pseudohirtipes*, *M. purpurascens* A. Costa, 1854) (Fig. 4). The clade containing members of the *M. hirtipes* group, including *M. microgemellata*, was recovered as monophyletic with 98% ultrafast bootstrap support.

Discussion

Since its description in 1965, no detailed records of *Mordellistena microgemellata* have been published, apart from mentions from Bulgaria, Cyprus, and Hungary by Horák (2020), all without further details. Even the type locality, "Graecia," is vague and may refer either to mainland Greece or to one of the many Greek islands in the eastern Mediterranean Sea. Although the first author has examined a substantial number of Mordellidae specimens from the Balkans, Greece, and Tunisia in the private collections and museums over the past years, no specimens of *M. microgemellata* were ever identified. During fieldwork in Tunisia in May 2025, eight localities in the northern and central regions of the country were sampled, but *M. microgemellata* was found only at a single locality near Bir Al Huffay. Based on the scarcity of available records, the species appears to be localised and rare.

In his original description, Ermisch (1965) assigned *Mordellistena microgemellata* to the *M. gemellata* species group based on the presence of two short lateral ctenidia on the metatibia. In his identification key to Central European *Mordellistena* (Ermisch 1977), however, *M. microgemellata* was erroneously characterised as having antennomeres 5–10 that are 1.5–2.0 times longer than wide. The value is incorrect, as both the holotype and the Tunisian specimens have antennomeres only 1.20–1.25 times longer than wide. Our observation also agrees with the original description, in which Ermisch noted that the antennomeres are "only slightly longer than wide, in female square." The distinctive structure of the male genitalia – particularly the left paramere, which has a long basal portion and a dorsal process strongly constricted medially and markedly expanded and obliquely truncate distally, and the right paramere, whose ventral process is shallowly bifurcate apically – allowed straightforward identification of the newly collected material after comparison with the *M. microgemellata* holotype.

Results of the COI barcoding region analyses suggest that *Mordellistena microgemellata* is not closely related to the species forming the *M. gemellata* clade but is instead nested within the *M. hirtipes* clade (Fig. 4). The close affinity of *M. microgemellata* to the *M. hirtipes* clade is further supported also by morphological characters, such as the expanded male second maxillary palpomere bearing strongly extended setae, the shape of male and female sternite VIII, and the structure of the parameres (Selnekovič and Kodada 2019). However, more comprehensive analyses based on larger portions of the genome are needed to test this hypothesis.

The females of *Mordellistena microgemellata* closely resemble the two female syntypes of *M. psammophila* Peyerimhoff, 1943, described from El Oued, Algeria, and not recorded since. The type locality of *M. psammophila* is situated approximately 275 km from Bir Al Huffay. However, differences in the shape of female sternite VIII and in the length of its sensilla confirm that these are two distinct species.

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Supplementary material 1

Sequences used in the analyses

Authors: Dávid Selnekovič, Ján Kodada, Enrico Ruzzier
Data type: xlsx
Explanation note: Accession numbers for sequences used in the analyses, with respective Barcoding Index Number and haplotype number.
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